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REQUIRED QUALITY CONTROL TESTS, QUALITY SPECIFICATIONS, AND SHIPPING PROCEDURES FOR LABORATORY PRODUCED MEDITERRANEAN FRUIT FLIES FOR STERILE INSECT CONTROL PROGRAMS

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Required Quality Control Tests, Quality Specifications, and Shipping Procedures for Laboratory Produced Mediterranean Fruit Flies for Sterile Insect Control Programs

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Introduction

This document is designed to provide standard procedures for required quality control tests and shipping methods as well as specifications for the flies to be used in sterile insect programs against the Mediterranean fruit fly, *Ceratitis capitata* (Wied.).

The information and procedures contained herein were developed in cooperation with the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). In addition to the references cited, the procedures are based upon work done at the Mediterranean fruit fly production/research laboratories located in Mexico, Guatemala, Hawaii, and Vienna, Austria. Copies of a draft of this document were circulated to persons concerned with fruit fly rearing, requesting comments and improvements. Suggestions received have been incorporated in this document.

(a) Quality Control Tests: The tests listed herein are required as indicated in order to ensure a good quality insect is being used in the program. Additional quality control tests have been developed and their use is optional. We feel these required tests will allow evaluation of fly quality during the conduct of an action program in the field.

(b) Specifications for Flies: A range of values, which is considered acceptable for a quality fly, is given for each quality control test. Departure from this range of values indicates a trend leading to reduced quality. If these specifications are not maintained, the flies will not be considered suitable for program use.

(c) Shipping Procedures: Three procedures are given which have been used in operational programs. Quality control data on flies shipped by each procedure indicate clearly that the pupae precooling method used in the Miami program in 1985 is superior. This will be the required shipping method if pupae are enroute 12 hours or longer.

Data Analysis, Presentation, and Communication

Establishing specifications for the quality of insects to be received by a project conducting a sterile insect release campaign is only the first step. Specifications have little value if the user cannot expect that they will be routinely met. That expectation requires an adequate quality control program at the production facility; a second requirement is analysis, verification, and communication between producer and user. Simple, routine procedures are available for collecting and preparing data on product quality that allow producer and user to evaluate the continuing processes of production and to predict trends leading to reduced quality. Among these are control charts.

The production facility will prepare a capability analysis of each of the variables identified in the list of required quality tests. That is, a sample of 50 measurements of each parameter will be accumulated and a mean value $\pm$ 3 Standard Deviations (SD) will be determined.

From this analysis, mean (X) and range (R) charts will be prepared. The daily or weekly measures of each test as production proceeds week by week will be presented on these charts. The charts will be provided to the user so that communication will be enhanced as to the impact of variations and trends of quality on fly quality after receipt by project user. Analysis and charting of each measured parameter at the receiving station will be similarly conducted to allow comparison*.

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* Computer analysis procedures are available from Dr. T. Ashley, ARS, Gainesville, FL 32602

Specifications for Flies Produced in the Laboratory:
Tests at the Fly Production Facility

Pre-Irradiation Tests

Pupal Size Test: See instructions, page 7.

Specifications: Ninety percent of the pupae fall within 1.67 mm to 1.86 mm with a mean of 1.76 mm in diameter (approximately 6 mg to 8 mg, mean of 7 mg in weight, respectively, is considered normal). When 25 percent of pupae average 1.72 mm (6.5 mg) or lower, a potential problem is indicated; and if continued over a period of 5-10 lots (days) will result in rejection of flies for use in the program. Run test daily.

Sex Ratio: See instructions, page 19.

Specifications: Variations of 45:55 percent of either sex are considered normal. A trend of 40:60 or greater indicates potential problems and possible rejection of the flies for program use. Run test weekly.

Percent Emergence: (The flies which eclose and escape the pupal case, puparia.) See instructions, page 10.

Specifications: Emergence in excess of 90 percent is considered acceptable. Trends down to 85 percent indicate potential problems and possible rejection. Emergence below 85 percent is not acceptable and flies will be rejected. Run test daily.

Percent Fliers: (The percentage of the test population, pupae, which fly out of the test chamber.) See instructions, page 10.

Specifications: When 80-90 percent of the pupae produce adults which can fly out of the test container, the population is considered normal. Trends down to 75-80 percent fliers indicate problems and possible rejection of flies. When less than 75 percent of the flies can fly, they are rejected. Run test daily.

Stress Test: (Longevity, with water, no food.) See instructions, page 18.

Specifications: Fifty percent mortality after 40-45 hours is acceptable. Consistent trends of greater than 50 percent mortality during this period would alert to a possible problem. Run test daily.

Mating Propensity Index: See instructions, page 12.

Specifications: This is a test of fundamental importance for the Sterile Insect Technique. It measures the capability of the sterile fly to mate and is an indication of mating capability with the wild fly population. The procedure described, herein,

is the best available at present. Yet, in the past, great variation in results was encountered from various production facilities and within individual facilities.

The most consistent mating propensity data we have experienced were that obtained during the Miami Medfly Eradication Program during May-July 1985. Tests were run both at the production facility in Hawaii and the release site in Miami. Indices for flies in Hawaii were approximately one-half those in Miami for the same batches of flies. Excluding the first shipment, indices in Miami ranged from 50 to 75. These results indicated a procedural problem which was identified as the holding procedure prior to release of both sexes in the mating chamber. Holding the flies in darkness for at least 10 hours prior to mating produced the most consistent and highest values.

Based upon these results, it is suggested that mean mating indices between 50 and 75 be considered satisfactory. Consistent values lower than this could lead to rejection of the flies for use in a program. Run tests at least weekly. Indications of loss of quality in other tests would trigger more frequent tests.

Post Irradiation Tests

Percent Emergence: (The flies which eclose and escape the pupal case, puparia.) See instructions, page 10.

Specifications: Emergence in excess of 85 percent is considered normal. Trends down to 80 percent emergence indicate a potential problem and possible rejection. Emergence below 80 percent would result in rejection of the flies for program use. Run test daily.

Percent Fliers: (The percentage of the test population, pupae, which escape or fly out of the test chamber.) See instructions, page 10.

Specifications: A range of 75 to 85 percent of the sample population flying out of the test container is considered satisfactory. Trends down to 70 percent fliers indicate potential problems and possible rejection. When less than 70 percent of the population can fly, they are rejected. Run test daily.

Specifications for Flies Produced in the Laboratory:
Tests at the Release Site

Percent Emergence: (The flies which eclose and escape the pupal case, puparia.) See instructions, page 10.

Specifications: Emergence above 80 percent is considered satisfactory. Trends down to 75 percent indicate potential problems and possible rejection. Emergence below 75 percent is unsatisfactory and flies will be rejected. Run test daily.

Percent Fliers: (The percentage of the test population, pupae, which escape or fly out of the test chamber.) See instructions, page 10.

Specifications: A range of 65 to 75 percent of the sample population, pupae, which escape or fly out of the test chamber is considered satisfactory. Trends down to 60 percent fliers indicate potential problems and possible rejection. When less than 60 percent of the population can fly, the flies are rejected for program use. Run test daily.

Mating Propensity Index: See instructions, page 12.

Specifications: Same as Mating Propensity Index under "Tests at the Fly Production Facility" except a mean mating index of 50 or greater should be considered satisfactory. Indices of 60 and above should be expected. Consistent values below 50 should be considered a warning of problems, and highly erratic values or values below 40 could lead to rejection of flies for program use.

Irradiation Verification

Specifications: A dosimetry label or similar device must be attached to each package of pupae which positively visually indicates the package of pupae has been exposed to radiation. A source of these labels is given in "Sources of Supplies." Packages of pupae will not be broken or repackaged after irradiation.

If so desired, a more elaborate system can be used which will give a readout of the level of radiation, the dosimetry label (and pupae) were subjected to.

Eggs in Sterile Females (optional)

This test is designed to determine if the production facility is irradiating pupae at the proper age. Females irradiated as late as 1 day prior to eclosion may contain viable eggs which would result in reproduction if mated with a normal male. From a sample of 100 pupae, dissect the first 10 females which emerge, and determine if eggs are present.

Required Quality Control Tests

During the last 10 years, various groups have attempted to develop a series of tests to compare the quality of Mediterranean fruit flies produced at different rearing laboratories. One of the first publications to bring together a large number of original papers and bibliographic references on quality control in fruit flies was "An Idea Book for Fruit Fly Workers" (Boller and Chambers 1977). This book suggested a group of tests designed to measure overall performance, individual traits, and laboratory production.

In 1979, the International Organization of Biological Control (IOBC) held a training course in Spain and published a reference manual (Calkins et al. 1979). This describes, in Spanish and English, tests to measure certain attributes in the laboratory and field. It included pupal size, flight ability and startle activity, olfactometry, mating propensity, dispersal and survival, and ratio tests.

In 1981, these procedures had been refined, given a name (RAPID) and published as "Measuring, Monitoring, and Improving the Quality of Mass-Reared Medflies" (Boller et al. 1981). This publication suggested that five tests, i.e., pupal size, flight ability, startle activity, response to pheromone, and mating propensity, be carried out at frequent intervals. Complete instructions are given on how to construct the apparatuses needed for the tests, methodology for carrying them out, and how international comparisons of quality profiles can be made.

In October 1981, an International Technical Group on Quality Control met in Guatemala to standardize basic methods and tests. They prepared a report listing six tests that should be carried out regularly and nine others as time and resources permit (Klee 1981). Procedures and samples of reporting forms to be used were given.

A complete list of quality control procedures for the laboratory and field has been published by the staff at the Medfly Rearing Facility in Metapa, Mexico (Orozco et al. 1983). This manual lists and explains more than 40 tests that can be used to extensively measure the quality of mass-produced flies.

The IOBC has sponsored two working groups on quality control (Gainesville-1982 and Wadensvile-1984) in which specialists from around the world have met to discuss, among other things, quality control tests. There appears to be almost unanimous agreement that, especially for Medflies, the RAPID system is to be used for international comparison of quality between production laboratories.

In 1984, USDA-ARS established a cooperative agreement with the Moscamed Commission in Guatemala to carry out a pilot test to promote systematic process for measuring, ensuring, and controlling quality through process control, production evaluation, and data management. The project scientists will use almost exclusively the RAPID system for laboratory and field tests.

Since the publication of the RAPID system, some constructive changes have been made in the equipment and procedures as used in various laboratories. Although the basic methodology is universally understood and practiced to varying degrees, there is still a need for exact standardization. Equally important will be the necessity to secure a meaningful commitment from the production and research facilities that they will recognize the validity of such a procedural document and use it. All of the various tests being used have their place in the overall monitoring of fly production, but for international comparisons of the basic quality parameters it has been decided to concentrate on three areas. The tests selected pupal size, emergence and flight, and mating propensity - can all be carried out with easily purchased or constructed equipment and require a minimum of laboratory space. Detailed instructions are given for preparing the apparatuses and standardizing the procedures. Sample forms are also provided for recording the results.

Required Quality Control Tests:
Pupal Size Test

Pupal size is usually expressed as average weight, and an arbitrary standard of ca. 7 mg has been accepted as optimum. This average weight is determined by taking random samples 2 days before emergence and weighing several lots of 100. The obvious problem associated with this is that not all rearing laboratories maintain the same conditions of temperature and relative humidity in the pupal holding rooms. Different relative humidities will lead to unequal rates of water loss during the entire pupal stage while different temperatures change the rate of maturity and, therefore, the chronological age at 2 days before emergence. Average pupal weight also does not give any indication of the size distribution, i.e., the percentage of pupal production falling into various groups, such as 6-7 mg range and 7-8 mg range, etc. To avoid these problems and ensure that a standard type of measurement is being made, size should be expressed as pupal diameter. As opposed to weight, pupal diameter does not change with age. If desired, it can be correlated with pupal weight and adult size.

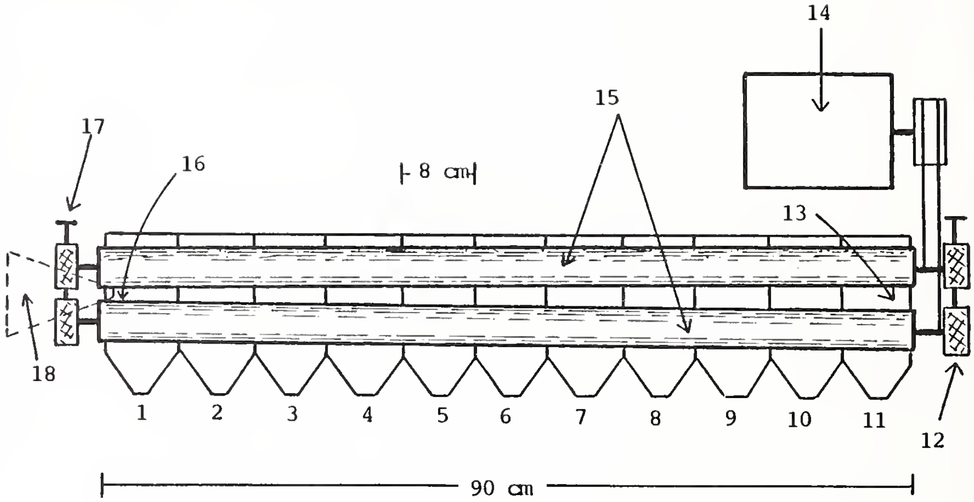
Equipment

A pupal sizing and separating machine is manufactured by a company in Vienna, Austria, and is available commercially. Several Medfly laboratories already have one in use, and others are being ordered. This device uses two sloping, side-by-side cylinders along which the pupae are moved. The revolving cylinders are aligned so that there is an increasing interspace between them through which the pupae will eventually fall as they move down the incline (Fig. 1). As illustrated, the cylinders can be adjusted to regulate the rate at which the interspace increases so that it is possible to collect the pupae into as many as 11 different size groups with #1 being the smallest and #11 the largest.

Technique

For standardization, the machine should be adjusted so that the narrowest distance between the cylinders is 1.4 mm and the greatest is 1.9 mm. The easiest way to calibrate these spaces is with automatic spark plug feeler gauges. These gauges are frequently expressed in fractions of an inch. In this case, the measurements are 0.055" and 0.075". This size setting was selected after experimenting with pupae produced at the laboratories in Metapa, Mexico, and Guatemala City, Guatemala. Using the calibration given, pupae that are 7 days old and fall into Group 7, will average between 6 and 7 mg. Table 1 gives the weight to size comparisons for 5 different days of the pupal period as measured at the Mexican and Guatemalan laboratories. This is presented to show not only how pupae of the same sizes can vary widely depending on the day they are weighed, but also how they can vary on the same day depending on the climatic conditions in the laboratory where they were produced.

(Fig. 1)



Pupal sizing and separating machine. 1 to 11 - Collecting troughs, 12 and 17 - Interspace adjusting screws, 13 - Lower interspace 1.9 mm or 0.075", 14 - Electric motor and pulleys, 15 - Revolving cylinders, 16 - Upper interspace 1.4 mm or 0.055", 18 - Pupal introduction funnel.

Table 1. Size group to weight relationships (in mg) of pupae produced in Guatemala (G) and Mexico (M). Pupae were sized and weighed from 1 to 9 days after larvae were collected.

		GROUPS									
		11	10	9	8	7	6	5	4	3	2
Day 1	G	10.4	9.5	9.3	8.8	8.2	7.7	7.4	7.2	6.0	4.6
	M	11.0	10.3	9.5	9.3	8.1	7.5	6.8	6.3	5.6	5.0
Day 3	G	8.8	8.0	7.5	7.2	6.6	6.2	6.0	5.2	4.7	4.5
	M	9.5	9.0	8.6	7.8	7.1	6.5	6.2	5.6	4.9	4.6
Day 5	G	7.9	7.7	7.4	6.9	6.4	6.1	5.8	5.1	4.5	4.4
	M	9.3	8.7	8.4	7.7	7.0	6.4	6.1	5.6	4.9	4.6
Day 7	G	8.0	7.3	7.0	6.5	6.0	5.5	5.2	4.7	4.2	3.9
	M	9.1	8.6	8.1	7.6	6.9	6.4	6.0	5.5	4.9	4.5
Day 9	G	8.0	7.3	6.9	6.5	6.0	5.5	5.2	4.5	4.2	3.7
	M	8.8	7.9	7.5	7.0	6.3	5.9	5.3	5.0	4.4	4.0

Obviously the lower relative humidity in Guatemala results in a much more rapid water loss in the pupae. These data support the use of the pupal sizing machine for comparison between various laboratories.

Before each use, the roller separation should be recalibrated. A random sample of ca. 500 pupae is selected from the lot to be measured and put through the sizing machine. Group 1 will collect debris but few, if any, larvae and can be discarded. As the pupae that have been collected in each group are counted, they should be examined and any that are stuck together or have debris attached to them, should be discarded. Form Q.C. 1 is then used to record the numbers in each group and calculate the size range by percentage. Run sample on day 7.

Required Quality Control Tests:
Percent Emergence and Flight Ability

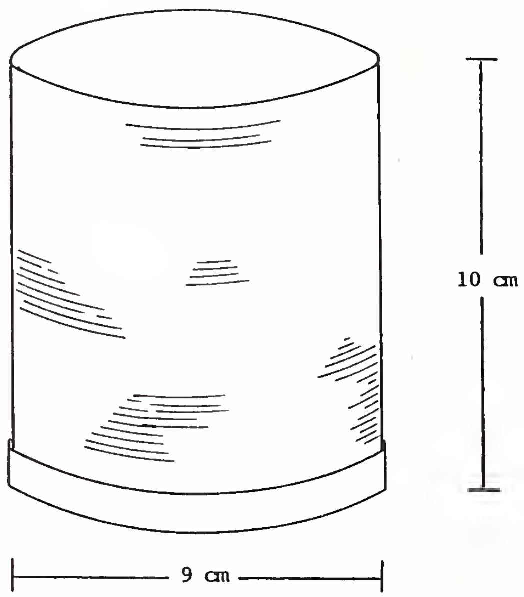
Discussion

Various types of containers and techniques have been used in the past for emergence and flight tests. Most of these have included tapping the side of the cup or tube to induce reluctant fliers to leave. This procedure is difficult to standardize - how long and hard to tap, so a system was developed whereby the flies must leave entirely on their own.

Equipment

The test apparatus consists of a petri dish and length of plexiglass pipe as diagramed in Fig. 2. The pipe should be painted black on the outside so that light will enter at the top only. Plexiglass has been chosen over cardboard or glass because it is unbreakable and can be washed and reused indefinitely.

(Fig. 2)



Flight ability tube. Plexiglass tubing, outside diameter 3 1/2" (8.9 cm) with 1/8" (3.175 mm) thick walls. Base is the lid of a standard 9-cm petri dish.

Technique

One to 2 days before emergence, 100 pupae are placed in the petri dish, and the flight tube is put in place. Before each use, the inside of the tube should be lightly coated with unscented talcum powder to prevent the flies from walking out. A 1 cm x 10 cm strip of paper folded accordionwise is placed against one edge of the cylinder in the emergence plate for a resting place for emerging flies. As the flies emerge, their only access to food and water would be to fly out of the tube.

One or more of these tubes can be put inside a larger collecting area such as a plexiglass cage. In this case, the fliers should be asperated out daily to keep them from falling back into one of the tubes when they die. The test is allowed to run until all the flies that emerged have escaped or died (2-3 days). When the test is completed, Form Q.C. 2 is used to record the necessary data from the material remaining in the petri dishes. Five replicates (of 100) are to be set up for each lot to be tested. Data on partially emerged and deformed flies can be used by the rearing staff to evaluate certain stress conditions, but for international comparisons, the two most important are percent emergence and percent fliers. The tubes and cages should be kept in a room maintained at 25 °C and ca. 60 percent relative humidity. The light level should average 1,500 lux with a 14-hour light, 10-hour dark photoperiod.

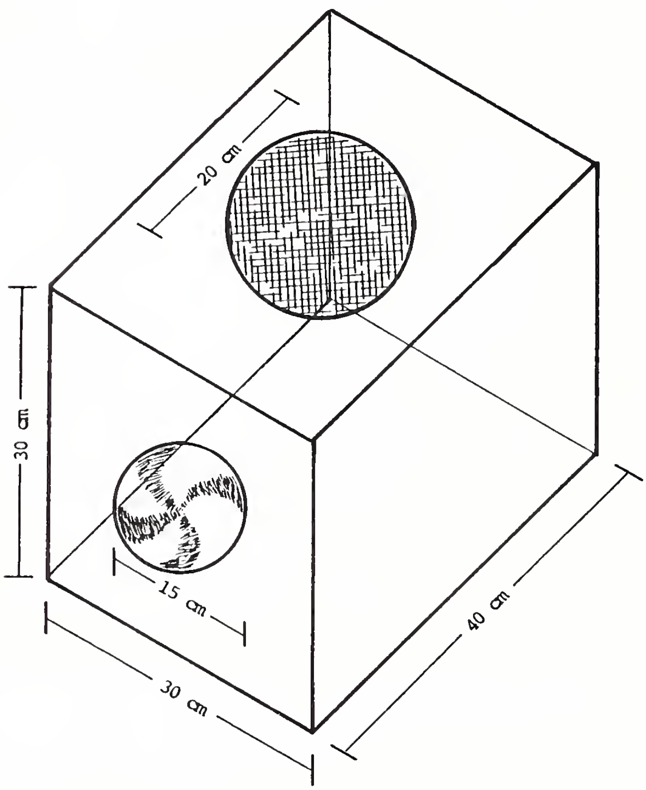
Required Quality Control Tests:
Mating Propensity Index

Discussion

This test is used principally to measure whether the male flies produced in a mass-rearing laboratory have retained the necessary traits to copulate with virgin females. The speed with which these matings take place under standardized conditions is used as a quantitative parameter. In the production facility, the crosses are usually fertile; while at the receiving end, they will be sterile to sterile unless field-collected females can be obtained.

When possible, sterile flies should be tested in the field with the wild fly population. Such tests done at 4- to 6-month intervals would provide a valuable reference for comparison with the more frequent tests done with laboratory-reared flies.

(Fig. 3)



Mating propensity cage. Made from 0.5 cm thick plexiglass. Top hole covered with 16 mesh screen and front sleeve made of fine nylon mesh. The best procedure to joining the edges of plexiglass sheets is to use methylene chloride.

Equipment

A plexiglass mating cage as diagramed in Fig. 3 is to be used. The sleeve should be made of a very light, fine mesh that will not create a shadow.

Technique

The test is set up by randomly collecting about 5,000 pupae from the lot to be evaluated just prior to irradiation at the production site, and immediately after receipt at the release site. The pupae are placed in emergence cages (plexiglass cages as described in Fig. 3) with food and water. Males and females are separated into appropriately labeled holding cages within an 8-hour period of emergence. Each individual cage should be stocked with 30 males or females. A total of six cages of each sex should be prepared. This will provide one extra cage since the test consists of five replicates. A sufficient number of each sex (180) should be collected within 8 hours. Any cages of females which are later found to contain one or more males must be discarded. Cages of males are inspected periodically and any females or mating pairs discovered are removed.

The sexes are held separately as described for 7 days; day 1 is the day emergence started. They should be maintained in a room with the following conditions: temperature 25 °C, relative humidity ca. 60 percent, and photoperiod of 14 hours light at about 1,500 lux and 10 hours of complete darkness. The test is begun immediately following at least 10 hours of darkness. Transfer 25 males and then 25 females within 5 minutes in subdued light to each mating cage. Prepared cage is then moved into full light and flies allowed to mate. It is very important that the cages have been washed with soap and water since the last use and that the test room has not been used to hold adult flies. The test should be carried out under the same climatic and light conditions for which the flies were held. When the mating cages have been prepared, a timer is started and pairs are removed as they mate. A record is kept of mating pairs which are removed by 10-minute intervals. After 60 minutes, the number of males and females not obviously deformed or crippled remaining in each cage is counted. The attached Form Q.C. 3 is then used to calculate the mating index.

FORM Q.C. 1

PUPAL SIZE

LOT NUMBER ____________________

DATE OF TEST __________________

COMMENTS:

GROUP	NUMBER COLLECTED	PERCENT TOTAL
2	______________	____________
3	______________	____________
4	______________	____________
5	______________	____________
6	______________	____________
7	______________	____________
8	______________	____________
9	______________	____________
10	______________	____________
11	______________	____________
TOTAL	______________	

FORM Q.C. 2 FLIGHT ABILITY AND EMERGENCE

LOT NUMBER ____________________

DATE OF TEST __________________

COMMENTS:

	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5
A. Unemerged Pupae	____	____	____	____	____
B. Halfemerged Pupae	____	____	____	____	____
C. Deformed Flies	____	____	____	____	____
D. Normal Flies	____	____	____	____	____
PERCENT EMERGENCE (100 minus A + B)	____	____	____	____	____
PERCENT FLIERS (100 minus A, B, C, & D)	____	____	____	____	____

AVERAGE PERCENT EMERGENCE ________________

AVERAGE PERCENT FLIERS ___________________

Instructions for FORM Q.C. 3

The test is divided into six 10-minute periods (A), and as mating pairs form in the cage, they are removed and recorded in column (B) as to when they mated. The number of pairs (B) for each period is multiplied by the weight factor (D) and entered as the index value (E). At the end of 60 minutes, any normal appearing, unmated flies remaining in the cage, are counted and the number of males or females, whichever is less, recorded as (F). This number when added to the number of accumulated pairs (C) will give the number of pairs (N) that could have participated in the test. This will usually be 25 but occasionally is less when flies have died or escaped during the test.

The Mating Index (MI) is calculated by totaling the index value (T) and dividing this sum by (N). The MI for the 5 replicates are then averaged to get a Mean Mating Index (MMI) for the lot being tested.

Example

(A)TIME	(B)# OF PAIRS	(C)ACCUM. PAIRS	(D)WEIGHT	(E)INDEX VALUE
0-10	7	7	100	700
10-20	8	15	50	400
20-30	5	20	33	165
30-40	2	22	25	50
40-50	1	23	20	20
50-60	0	23	15	0
	F = 1	N = 24	1	1
				T = 1,336

$$\frac{1,336}{24} = 55.66$$

REPETITION NO. ______________

MATING INDEX ______________

FORM Q.C. 3 MATING PROPENSITY

LOT NUMBER ____________________

DATE OF TEST ____________________

COMMENTS:

(A)TIME	(B)# OF PAIRS	(C)ACCUM. PAIRS	(D)WEIGHT	(E)INDEX VALUE
0-10			100	
10-20			50	
20-30			33	
30-40			25	
40-50			20	
50-60			15	
	F =	N =	1	
				T =

REPETITION NO. ________________

MATING INDEX ________________

Required Quality Control Tests:
Stress Test

The longevity of adults under stress (water but no food) is an indicator of fly quality. Tests should be done daily.

Procedure

Fifty females and 50 males recently emerged are placed in plexiglass cages with water (five replications). The cages should be held at 26 °C and 60-70 percent relative humidity. Every 8 hours, count the number of individuals that have died and calculate the average length of life based on the number of dead flies. When 50 percent of the flies are dead, the test is terminated. The results are expressed in hours to 50 percent mortality.

Materials and Equipment

1. Acrylic cages
2. Tweezers

Required Quality Control Tests:
Sex Ratio

Significant deviation in the 50:50 sex ratio in the fly population may give an early indication of problems. These problems could be of a genetic nature or possible procedural effects. Test is done weekly.

Procedure

Take five samples of 100 pupae each and place in petri dishes. These are held in a bioclimatic chamber at 26 °C and 60 percent relative humidity. Four days after emergence, the existing population is counted for each sex and the following ratio is made.

$$\text{Female-Male Ratio} = \frac{\text{No. Males}}{\text{No. Females}}$$

Materials and Supplies

1. Petri dishes
2. Manual counter
3. Stereoscopic microscope
4. Tweezers

Shipping Procedures:
Shipping Procedures Prior to 1984

Two basic methods have been used prior to 1984 to ship sterile Medfly pupae from the various rearing laboratories to the location where they are emerged and released in an integrated control program. These include:

1. Plastic airtight bottles with no means provided to control temperature of pupae enroute.

2. Boxes of various types with pupae in airtight containers and provisions for holding temperature down inside the box.

Pupae from the Mexico-USDA laboratory in Metapa, Mexico, are irradiated and shipped in 10 or 15 liter plastic bottles while those at the two rearing labs in Hawaii (USDA/ARS and California Medfly Lab) are irradiated in one type of container and then transferred to plastic bags for shipment in styrofoam or cardboard boxes. Sources for these materials are given under "Sources of Supplies." Both methods of shipment result in a state of hypoxia developing in the containers.

Plastic Bottles

During the last 6-7 days of maturation, the pupae are kept at 25 ± 1 °C, and then 1-2 days before emergence, they are dyed with flourescent powder and packed in 10 liter widemouth polyethylene bottles. The bottles are completely filled with pupae and the cap threads smeared with petroleum jelly to create an airtight seal. The bottles are then left in a room at 18-20 °C for a minimum of 1 hour before irradiation to allow the pupae to exhaust the oxygen supply. After irradiation, the bottles are sent in a refrigerated van, either to the local dispersion center or to the airport for out-of-country shipment. No insulating material is used around the bottles at any time.

The Metapa lab has supplied pupae to Guatemala for many years, and also from October 1980 to July 1981, approximately 100 million per week were sent to California. Both shipments traveled together by charter airplane to Guatemala City where the California allotment was put on a commercial flight. Table 2 gives the comparative quality control records for Mexico, Guatemala, and California during this period. All pupae were from the same batches and handled the same up until the time some were shipped to Guatemala and California.

Styrofoam and Cardboard Boxes

Hawaii to California (USDA/ARS) - The USDA/ARS laboratory in Hawaii irradiated Medfly pupae in a high nitrogen atmosphere, containing approximately 0.03 percent oxygen, with gamma radiation from a Cobalt-60 source. The irradiated pupae were mixed thoroughly with a marking dye and packaged in polyethylene bags for shipment in a styrofoam ice chest.

Table 2. Quality control data on pupae produced at Metapa, Mexico, and shipped to three different emergence centers in polyethylene bottles.

Quality Control Center	Average Hours in Anoxia	Average Percent Emergence	Average Percent Fliers
Tapachula, Mexico	4	84.8	74.2
Guatemala City	8	82.1	68.8
Los Gatos, CA	20	77.6	66.5

The thickness, diameter, and length of the polyethylene bags were 1.5 mil, 8.3 cm, and 76 cm, respectively. Three liters of dye-marked pupae were placed in each bag. The open end was twisted tightly against the pupae and secured with a rubber band. A total of six bags was prepared for each ice chest.

The ice chest was modified slightly and provided with six spacers and two shelves with 2.2 cm holes. The wall thickness and inside dimensions of the ice chest were 2.5 cm and 65.6 x 29.8 x 25 cm, respectively. The dimensions of the spacer and the shelf were 60.6 x 1.3 x 8.6 cm and 60.6 x 1.9 x 2.5 cm, respectively. Shelf-A had 26 holes. Four blocks, 7.8 x 1.3 x 2.4 cm, were glued on the top side to restrain the bags of coolant gel and the shelf when the ice chest was closed. Shelf-B had 32 holes and rested on six supports (6.4 x 1.3 x 8.6 cm) glued on the longer sides next to the bottom of the chest.

The loading of the ice chest started from the bottom as follows:

1. Three bags of pupae with spacer between each bag.

2. Shelf-B on the supports and spacers.

3. Three bags of pupae on Shelf-B with a spacer between each bag and between the side and the bag.

4. Shelf-A on the spacers.

5. Six 9-oz bags of coolant gel on Shelf-A. The air temperature in the closed ice chest was 10-21 °C during 20 hours at room temperatures of 25 °C.

Hawaii to California and Guatemala (California/Hawaii Medfly Rearing Lab) - From May 1981 through July 1982 the California/Hawaii Medfly Project Lab airshipped more than 4 billion irradiated, dyed Mediterranean fruit fly pupae to California and to Guatemala, Central America.

The shipping method utilized was essentially that developed by the USDA/ARS laboratory in Hawaii. Some modifications were necessary because of the greater shipment size.

Description of Shipping Container - Construction is of double-walled corrugated cardboard (300 lb test BA flute) measuring 70 x 32.2 x 26.7 cm with a top and bottom full overlap. The inner wall is treated with Michelman Coating, a water resistant compound. A 68.4 x 30.6 x 26.7 cm liner (300 lb test A flute) is fitted inside the container. Polybags filled with pupae are separated with 28.8 x 65 cm vented shelving (150 lb test A flute) which is supported by cardboard spacers glued to both sides of the container. The disposable shipping container holds approximately 36 liters or 2.34 million pupae.

Procedure - Pupae placed in 46.2 x 38.7 x 2.1 cm irradiation trays were chilled until pupal temperature dropped from 20-26.7 °C (temperature depending on pupal holding schedule the previous night) to 12.7 °C. Otherwise pupal temperature may reach as high as 35 °C while being held for the 3-hour irradiation process, usually in batches of ca. 15.5 million pupae (240 liters). Before packing, pupae were again chilled to 20 °C, then dyed and packed in 1.5 mil polyethylene bags (90 x 7.5 cm with 5 cm gusset). Three layers of three polybags, each with a 4-liter capacity (ca. 260,000 pupae), were placed in each shipping container. A set of four blocks of blue ice was placed between the top two layers.

Shipping time (time of final packing to time containers opened) to California from Hawaii averaged 19 hours while to Guatemala, Central America, averaged 41 hours.

Bottles versus Boxes

Mexico to California - In November 1980, shipments from Tapachula, Mexico, to California used both systems. Some boxes were packed with blue ice and some without. Pupae were packed and irradiated very early in the morning and sent by charter flight to Guatemala City. Program personnel met the airplane and delivered the bottles and boxes to the cargo handling department of a commercial flight leaving for Los Angeles. This flight was met on arrival at the airport and the pupae delivered to the emergence center. Time in hypoxia was usually about 18 hours with little chance of exposure to adverse conditions during shipping. Table 3 gives quality control comparisons.

Table 3. Quality control data on pupae produced at Metapa, Mexico, and sent to Los Gatos, California, in bottles and boxes with and without blue ice.

	BOTTLES	BOXES	
		NO ICE	WITH ICE
Percent emergence	76.7	82.5	82.3
Percent fliers	69.5	68.9	75.9

Hawaii to Guatemala - During August and September of 1982, a series of tests were conducted in which simultaneous shipments were made using both systems to deliver pupae from Hawaii to Guatemala. Pupae were irradiated during midmorning of day 1 and shipped that afternoon to Los Angeles. They changed airplanes and arrived in Guatemala late in the evening on day 2. The pupae could not be cleared through Customs until the morning of day 3, which meant they were in hypoxia for about 40 hours. It was also not possible to observe how the shipping containers were stored and handled during the layover in Los Angeles. Table 4 gives the quality control results for this test and it is obvious the boxes gave superior protection during shipment.

Table 4. Quality control data on containers used to ship pupae from Hawaii to Guatemala.

	BOTTLES	BOXES
Percent emergence	48.4	73.1
Percent fliers	33.7	61.7

Although there seemed to be little difference in the performance of flies from pupae shipped in either bottles or boxes when the shipping and handling were closely monitored by program personnel, consideration should be given to possible intransit physical and environmental conditions.

The plastic bottles are extremely durable and can be used repeatedly for years. They also provide a reasonable amount of insulation but are not as good as the boxes, should temperature extremes be encountered. It is probable that each individual Medfly project will want to analyze their particular situation and possibly test each system before making a final decision.

Shipping Procedures:
Packing, Irradiating, and Shipping Procedures Developed 1984/85 and used to Ship Sterile Pupae from Honolulu to Miami for the 1985 Medfly Eradication Program in Florida

Pupae identified to be 2 days from adult eclosion (eye color - dark brown) are precooled to ca. 15 °C. They are then dyed and filled into polybags (10.2 x 7.6 x 76.2 cm, 1.5 mil thick) for irradiation and shipment. (Note: Polybags sent to Miami were not filled to the capacity of the irradiation canisters, and the bags were tied with extra headspace to allow the pupae to be redistributed so three layers of polybags could be loaded into the existing 35.6 cm deep shipping cartons. A fully filled polybag measures 12.7 cm in diameter.) Radiation-sensitive tapes are placed into each bag of pupae before the open ends are twisted and tied tightly into airtight knots. The air space between the pupae and the knots should be kept to a minimum. To create the desired hypoxic atmosphere within the bags, they are allowed to stand for a minimum of 1 hour before they are irradiated. The room temperature should be maintained at the pupal precooling temperature at all times.

For irradiation, the polybags with pupae are inserted into canisters and cycled through the Husman irradiator. The bags are serially tagged as they are removed from the canisters after irradiation. At the same time, USDA, APHIS, baggage inspection tapes are applied over the tied ends of the bags to form security seals. These bags are then immediately loaded into cardboard shipping containers. The lids are closed with hot glue and are additionally secured with filament tapes around the girth of the boxes. The cartons are stored in a 7 °C reefer while waiting for air transportation. While in transit, arrangements should be made to have the cartons refrigerated if there are long layovers or periods of extreme high ambient temperature. To alert airline personnel, each carton should be conspicuously labeled either "REFRIGERATE" or "CHILL."

Typical Time Schedule for Packing, Irradiating, and Shipping Medfly Pupae to Miami
(27 polybags in 3 shipping cartons - ca. 6.5 million pupae)

1. Precooling of pupae to ca. 18 °C	10:00 am - 11:00 am
2. Dyeing and bagging of pupae	11:00 am - 12:00 pm
3. Holding period for hypoxia	12:00 pm - 1:00 pm
4. Irradiation and packaging	1:00 pm - 2:45 pm
5. Storage in 7 °C reefer while waiting to be transported to airport	3:00 pm - 6:00 pm
6. ETD Honolulu - Western #566	9:50 pm
ETA Los Angeles	5:50 am (next morning)
7. ETD Los Angeles - Eastern #518	8:15 am
ETA Miami	4:02 pm
8. Arrival at Medfly Project	5:00 pm

Total Elapsed Time ----------- ca. 26 hours
Total Time in Polybag --------- ca. 24 hours

This procedure resulted in sterile flies at the release site in essentially the same condition as those emerged at the production facility, based upon quality control tests at both locations.

This procedure, which entails precooling pupae 15 °C before packing and irradiation, is recommended for shipments of 12 hours or longer.

Sources of Supplies

- Cardboard Boxes

  Weyerhaeuser Company
  900 North Nimitz Highway
  Honolulu, HI 96817

- Dosimetry Labels

  Allied Corporation
  New Ventures Group
  P.O. Box 1021 R
  Morristown, NJ 07960

  Code #96012800-96

- Plastic Bags

  Atlantic Poly, Inc.
  672 Pleasant Street
  Norwood, MA 02062

  4" x 3" x 24" Plastic Bag
  Gusseted Design, Clear Polyethylene
  2 mil thickness
  $27.30 per 1,000

- Plastic Bottles (10 liter)

  Consolidated Plastics Company
  9085 Freeway Drive
  Macedonia, OH 44056

  Catalog #2234-0020
  $102.55 per case of 6

- Plexiglass Tubing

  Consolidated Plastics Company
  9085 Freeway Drive
  Macedonia, OH 44056

  Order: Tube-Pak, size No. 52D
  I.D. 3 1/4, O.D. 3 1/2, 8 ft length
  Part No. 86048C. $53.78 each

- Pupal Sizing and Separating Machine (Commercial name is Puppentrennmaschine mit 2 Walzenpaaren)

  Ing. Kroneis, Inglaseegasse
  30-32, A-1191
  Vienna, Austria

- Styrofoam Boxes

  Pacific Allied Products
  44-157 Nanamoana Street
  Kaneohe, HI 96744

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